Gastric secretion does not affect the reliability of the (13) C-acetate breath test: A validation of the (13) C-acetate breath test by magnetic resonance imaging

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Abstract: Background (13) C-Acetate labeled meals are widely used to determine meal emptying by means of analyzing resulting (13) CO(2) exhalation dynamics. In contrast to the underlying metabolic processes, only few (13) C breath test meal emptying studies have focused on intragastric processes that may alter (13) CO(2) exhalation. This work assessed the effect of enhanced gastric secretion on the reliability of half emptying time (t50) measurements by (13) C-acetate breath test. Methods (13) CO(2) exhalation data were acquired in a double-blind, randomized, cross-over gastric emptying study in 12 healthy volunteers receiving either pentagastrin or placebo intravenously. The standard method proposed by Ghoos et al. was applied to calculate t50 ($t50_{Ghoos}$) $from(13)CO(2)exhalationdata, whichwerecomparedandtestedforagreementtome$ 0.67), $however, apositiveoffsetof136minfort50_{Ghoos}.Nocorrelationwasdetectedbetween AUC\_SV(60)andcPDR(60)(r$ 0.11). $Both, breathtestandMRI, revealedaprolongedt50underpentagastrininfusionwithmediandifferencesint50_{Ghooso}$ $84]min(P = 0.002)andt50_{M}Vof39[28-52]min(P = 0.002).ConclusionsInferencesThisstudysuggeststhat(13)CO(2)exh$ $labeledliquidtestmealisnotaffectedbystimulatedgastricsecretion, butisratherreflectingthedynamicsofmealorcaloricen$

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Running title: Effect of gastric secretion on breath test

Abbreviations

MRI: Magnetic resonance imaging

plc: Placebo

pgs: Pentagastrin

IV: intravenous(ly)

t50: Half emptying time

t50_Ghoos: Breath test derived gastric content half emptying time [min]

t50_MV: MRI derived gastric meal half emptying time [min]

PDR: Percentage dose recovered [% h^{-1}]

cPDR: Cumulative percentage dose recovered [%]

GCV: Gastric content volume [mL]

MV: Meal volume [mL]

SV: Secretion volume [mL]

CA: Contrast agent

AUC_SV_{60}: Area under the curve of secretion volume during infusion period [mL*min]

Abstract

Background:

^{13}C-Acetate labeled meals are widely used to determine meal emptying by means of analyzing resulting $^{13}CO_2$ exhalation dynamics. In contrast to the underlying metabolic processes, only few ^{13}C breath test meal emptying studies have focused on intragastric processes that may alter $^{13}CO_2$ exhalation. This work assessed the effect of enhanced gastric secretion on the reliability of half emptying time (t50) measurements by ^{13}C-acetate breath test.

Methods:

$^{13}CO_2$ exhalation data was acquired in a double-blind, randomized, crossover gastric emptying study in 12 healthy volunteers receiving either pentagastrin or placebo intravenously. The standard method proposed by Ghoos et al. was applied to calculate t50 (t50_Ghoos) from $^{13}CO_2$ exhalation data, which was compared and tested for agreement to meal half emptying times (t50_MV) from concurrent recorded MRI volume data. In addition, the accumulated gastric secretion volumes during infusion as detected by MRI (AUC_SV_{60}) were correlated to the corresponding cumulative percent ^{13}C doses recovered ($cPDR_{60}$).

Key Results:

t50_Ghoos and t50_MV showed a linear correlation with a slope of 1.1±0.3 (r^2=.67), however, a positive offset of 136 min for t50_Ghoos. No correlation was detected between AUC_SV_{60} and $cPDR_{60}$ (r^2=.11). Both, breath test and MRI, revealed a prolonged t50 under pentagastrin infusion with median differences in t50_Ghoos of 45[28-84] min (P=.002) and t50_MV of 39[28-52] min (P=.002).

Conclusions & Interferences:

This study suggests that $^{13}CO_2$ exhalation after ingestion of a ^{13}C-labeled liquid test meal is not affected by stimulated gastric secretion, but is rather reflecting the dynamics of meal or caloric emptying from the stomach.

Keywords:

^{13}C-acetate breath test; gastric secretion; gastric emptying; magnetic resonance imaging

Introduction

Breath tests by means of stable ^{13}C-isotopes have increasingly gained importance in hepatic and gastroenterological functional diagnostics. They are attractive, non-invasive and multifunctional tools used for different clinical applications such as the quantification of organ functions or the determination of gastrointestinal transport.(1)

To measure gastric emptying, breath tests with ^{13}C labeled fatty acids, such as ^{13}C-acetate, ^{13}C-octanoic acid or naturally ^{13}C-enriched *Spirulina platensis* have been proposed to replace radioscintigraphy, which is still the gold standard.(2) Ingested ^{13}C-substrates are emptied from the stomach, absorbed in the small intestine, undergo catabolism in the liver, enter the body's bicarbonate pool, and then are excreted as $^{13}CO_2$ in the breath, where ^{13}C can finally be detected.(2) Assuming that all these steps are intact, the rate-limiting step in this process is the gastric emptying. Validation studies have shown, that the performance of breath tests is not affected by variation in hepatic fatty acid oxidation(3, 4) or metabolic and absorptive changes in critical disease, diabetes mellitus, liver cirrhosis and Crohn`s disease.(5) Further, it has been demonstrated that intestinal marker delivery is accurately and promptly reflected by $^{13}CO_2$ exhalation and that $^{13}CO_2$ formation is not influenced by calorie dependent digestive processes.(6) In contrast, the ^{13}C breath test is not fully validated for intragastric influences on ^{13}C-marker metabolism or distribution that could lead to a misinterpretation of gastric emptying measurements. Gastric secretion is stimulated by nutrient intake and known to influence gastric emptying.(8) Large amounts of gastric secretion may conceivably lead to dilution and redistribution of the ^{13}C-acetate within the test meal, presumably resulting in alterations of $^{13}CO_2$ exhalation and thus systematic errors in computed t50. Currently, magnetic resonance imaging (MRI) is the only non-invasive gastric emptying measurement method to separate meal and secretion from overall gastric content and thus to quantify meal emptying, gastric secretion volume and related intragastric distribution and mixing.(6, 9-11)

This work aimed to investigate the effect of enhanced gastric secretion on the reliability of meal emptying measurements by ^{13}C-acetate breath test. To this end, concurrent

measurements of ^{13}C exhalation data and of MRI volume data of gastric secretion and meal from a recent randomized placebo-controlled cross-over study in subjects with and without pentagastrin stimulated gastric secretion were analyzed and compared.

Methods

Ethics

This analysis used data acquired during a recently published study by Goetze et al.(12) The study was carried out according to Good Clinical Practice and the Declaration of Helsinki. The study protocol was approved by the local Ethics Committee and the Swiss national agency for therapeutic products (registration numbers 1152 and 2005dr2207). All measurements were completed without complications or adverse events.

Test meal

A sterilized glucose solution (500 mL, 200 kcal; Fresenius Kabi AG, Stans, Switzerland) was mixed homogeneously with 5 g locust bean gum powder (Rapunzel Naturkost AG, Legau, Germany). The test meal was labelled with 1200 µmol L^{-1} of the paramagnetic contrast agent Gadolinium-DOTA (Dotarem®, Laboratoire Guerbet, Roissy CDG, France) and 200 mg L^{-1} of [1-^{13}C] sodium acetate (Euriso-Top, Saint-Aubin Cedex, France). The mixture was ingested at 37 °C, where it had a viscosity of 202 mPa s^{-1} and an osmolality of 505 mOsm L^{-1}.

Volunteers and study design

Primary outcome of this work was the difference in meal emptying half time between ^{13}C-acetate breath test and MRI. The sample size of 12 participants was determined based on the study of Treier et al.(13), a two-sided 5% significance level and a power of 90% allowing for the detection of a difference of 15 mL in gastric secretion volume. Eligible participants were healthy adults aged 18 - 55, recruited via public announcement. Exclusion criteria were pregnancy and lactation, previous abdominal surgery (except appendectomy), intake of any medications apart from oral contraceptives, significant abnormality on a 12-lead ECG, claustrophobia and presence of metallic implants, devices or foreign bodies. Twelve healthy volunteers (seven male) with a mean age of 28 years (22 - 34 years) and a mean body mass index of 23.2 kg m^{-2} (20.3 - 28.0 kg m^{-2}) were investigated between February and May 2006

in a single center, double-blind, randomized, placebo-controlled and cross-over study design. Randomization was assured by a computer generated random list and stratified with a 1:1 allocation using random block sizes of 6. The two study days were separated by 2 - 14 days and each subject was investigated after an 8 h fasting period. Either pentagastrin (pgs; 0.6 µg kg^{-1} h^{-1}; Cambridge Laboratories, Wallsend, UK) or placebo (plc; sodium chloride 0.9% Baxter®; Baxter, Volketswil, Switzerland), was infused intravenously (IV) at time point t=0 min for 60 minutes. Volunteers ingested the test meal within 3 - 5 min after IV infusion was started. Breath samples were taken in fasting state and then after meal intake at regular time intervals until 180 min. Concurrent MRI measurements were performed until 90 minutes after meal intake. Heart rate and blood pressure were monitored over the whole study period.

^{13}C-Acetate breath test and MRI data

To focus on the effect of stimulated gastric secretion on $^{13}CO_2$ excretion, this work analyzed breath test and MRI data during the pentagastrin study arm only for the IV infusion period, i.e. until 60 min. After the end of pentagastrin infusion, MRI volume data showed a change in the dynamics of secretion and also for meal emptying and, therefore, standard analytical methods could no longer be applied for the entire dataset.

^{13}C-Acetate breath test data $^{13}CO_2/^{12}CO_2$ ratios were determined by molecular correlation spectroscopy (BreathID, Exalenz, Jerusalem, Israel) approximately once every 3 min with an automatic nasal breath sampling device under continuous capnographic control. The results were expressed as delta (δ) value per mil and delta over baseline ($DOB_t=\delta_{Sample}-\delta_0$).(14) DOB_t was used to determine the percentage dose of ^{13}C recovered (PDR [% h^{-1}]) and the cumulative PDR (cPDR [%]) at each measurement time point until the end of analysis period and if indicated as $cPDR_{60}$ until the end of IV infusion period.(14-16) According to the standard breath test analysis methods described by Ghoos et al.(14), meal emptying was quantified from the cPDR data by computing the parameter t50 (i.e. half emptying time) with $t50\_Ghoos = (-1*k^{-1})*\ln(1-2^{-1/\beta})$ using the model $cPDR(t) = m*[1-e^{(-k\cdot t)}]^{\beta}$. Using nonlinear

mixed-effect modeling, k and β were determined per subject and per visit whereas m was estimated for the population.

MRI data Magnetic resonance imaging measurements were performed in supine body position approximately once every 10 min on a whole-body MRI system (1.5 T Achieva; Philips Medical Systems, Best, The Netherlands). Imaging techniques were described previously.(6) The secretion volume (SV) and the contrast agent (CA) labelled meal volume (MV) were extracted from the previously determined gastric content volume (GCV) according to an established and validated procedure.(10, 11) In brief, based on MRI T1 mapping data of the GCV and a "calibration curve" that describes the interrelation between the measured intragastric T1 values and the CA concentration in MV, the percentage meal volume (%meal) was determined for each MR image series recorded. Based on the derived %meal, secretion and meal volume were then calculated as MV = GCV*%meal and SV = GCV-MV, respectively. Since MV did not show a lag effect, MV data was fitted using a power-exponential mixed-effect model, $MV(t) = MV_0 * e^{-(t/tempt)}$. To allow the comparison with the breath data that reflects the emptying of the ^{13}C labeled meal, the half emptying time of MV, $t50\_MV = t_{empt} * \ln(2)$, was calculated. In addition, the area under the curve of SV until end of IV infusion period (AUC_SV_{60}) was calculated by the trapezoid method.

Statistics

Data processing, statistical analyses and plots were done by R 2.13.1 (R Foundation for Statistical Computing, Vienna, Austria) and GraphPad Prism® 5. Nonlinear mixed-effect modeling was performed using the *nlme* function of R.(17) A linear regression model was used to test for correlation between t50 values derived from breath test and MRI data, and agreement between t50 values visualized by the Bland-Altman plot. All *nlme* and linear regression parameter estimates are given as value ± standard error. Effects of pentagastrin infusion on half emptying times were compared using paired Wilcoxon signed-rank test and differences in t50 between the study arms given as median [95% CI]. Correlation between

AUC_SV_{60} and $cPDR_{60}$ was determined by linear regression analysis. The significance level was set to P=.05.

Results

Breath test and MRI data

Visual inspection of the data revealed that $^{13}CO_2$ exhalation measurements, expressed as cPDR and PDR, were lower during pentagastrin infusion compared to placebo in the majority of the volunteers (**figure 1A**). Also, concurrent meal volume decrease, as assessed by MRI, appeared to be slower and secretion volume larger during pentagastrin infusion compared to placebo (**figure 1B**). Both, breath test and MRI data, showed inter-individual variations during both placebo and pentagastrin infusion.

Fits of cPDR and MV overlaid on the original data are presented in **figure 2 A** and **B**, respectively. Individual estimates of model parameters are listed in the supplemental material **table S1**.

Correlation and agreement of breath test and MRI data

Due to technical difficulties during pentagastrin treatment, breath test data of one volunteer was missing and excluded from the analyses.

Treatment independent, i.e. all data encompassing, half emptying times derived from breath test (t50_Ghoos) and treatment independent meal half emptying times derived from MRI (t50_MV) showed a linear correlation with a slope of 1.1±0.3 and r^2 =.67 (**figure 3A**). The Bland-Altman plot revealed a positive offset of 136 min for t50_Ghoos compared to t50_MV (**figure 3B**). No correlation (r^2 =.11) was detected between all data encompassing AUC_SV_{60} and $cPDR_{60}$ (**figure 3C**) suggesting a negligible influence of gastric secretion on $^{13}CO_2$ exhalation.

Effect of pentagastrin stimulation on gastric meal emptying half time

Breath test and MRI data both revealed a longer t50 for the pentagastrin study arm (**figure 4A**). **Table 1** shows the individual differences in t50 between pentagastrin and placebo.

Median [95% CI] differences in t50_Ghoos and t50_MV were 45 min [28-84], P=.002 and 39 min [28-52], P=.002, respectively (**figure 4B**).

Discussion

To investigate the effect of enhanced gastric secretion on the reliability of meal emptying measurements by ^{13}C-acetate breath test, this work analyzed simultaneously acquired ^{13}C-acetate breath test data and meal emptying data recorded by MRI. Independent of the amount of gastric secretion, a linear correlation for the derived half emptying times (t50) was found between both methods and, compared to MRI, a large positive offset in t50 was determined for the ^{13}C breath test data analyzed by the method proposed by Ghoos et al.(14) As expected, pentagastrin caused a prolongation in meal half emptying times (12, 18), that was detected with both methods. The prolongation in ^{13}C-acetate breath test half emptying time observed during pentagastrin infusion was comparable to the prolongation detected by MRI, further suggesting no confounding effect of gastric secretion on $^{13}CO_2$ exhalation data.

Currently, MRI is the only method to non-invasively assess gastric secretion volume and related distribution and mixing of gastric secretion.(6, 9-11) The applied quantitative MRI technique(6, 13) enables the extraction of meal and secretion volume from imaged gastric content volume, thus allowing a separate quantification of meal emptying from and secretion production into the stomach. MRI detected an onset of different meal emptying and gastric secretion dynamics after the end of the pentagastrin infusion period (data not presented here). This characteristic of the meal emptying prevented incorporation of the entire dataset of the pentagastrin study arm into a standard non-linear mixed effect model analysis. Therefore, as already pointed out, analysis of data from the pentagastrin study arm was limited to the pentagastrin infusion period. Data from MRI revealed that gastric secretion volume was increased by pentagastrin infusion with inter-individual differences. From visual inspection of MRI data, the intended secretion stimulating effect was detectable within the first 20-30 min after start of infusion (**figure 1B**), which is in accordance with past findings, where an increase in gastric acid output after stimulation with gastrin derivates was seen after a comparable period.(19, 20)

In all subjects, the detected cumulative $^{13}CO_2$ exhalation dynamic was more related to the meal emptying dynamics than to the integrated volume of gastric secretion (**figure 3**). Breath test derived half emptying times (t50_Ghoos) were well correlated with the MRI derived meal half emptying times (t50_MV) (**figure 3A**), however, exhibited a considerable positive offset of 136 min in the Bland-Altman plot (**figure 3B**).

Positive offsets in t50 derived by the ^{13}C-acetate breath test have previously been described in other comparative imaging studies using ultrasonography and gamma-scintigraphy.(21, 22) Using the method proposed by Ghoos et al. (14), these studies found mean offsets of 41 min and 49 min, respectively, in comparable test meals. The authors related their observed offset to all required post-gastric processing steps of the ^{13}C-acetate marker preceding $^{13}CO_2$ exhalation. In studies comparing ^{13}C-octanoate breath test with gamma-scintigraphy, Lee et al.(23) and Choi et al.(24) reported mean positive offsets in emptying half times derived from 4-hour ^{13}C breath test data of 144 and 69 min, respectively. These studies concluded that the offset can partly be assigned to an overestimation of the parameter m which is used for calculation of the t50_Ghoos. This parameter m is equivalent to the maximal value of cPDR, i.e. cPDR(∞), and is dependent on the actual shape of the cPDR curve, particularly if a plateau in $^{13}CO_2$ exhalation data has not been reached during the acquisition period. The parameter m usually approximates 40-80% of the ingested ^{13}C amount, depending on the irreversible non-pulmonary loss of the ^{13}C-atom.(23-25) The limited time window available for the analysis during the pentagastrin study arm did not allow for individual estimation of the parameter m in the majority of subjects. Therefore, m was determined for the population, whereas the parameters k and β were simultaneously fitted for each individual. This represents a valid and common approach in pharmacometrics (26, 27) and was here performed using the *nlme* function of the program R. k (the elimination constant of the ^{13}C atom in breath) and β (the steepness of the rise of $^{13}CO_2$ exhalation) depend on the type of gastric emptying (e.g. if it is fast, slow). The parameter m depends directly on fixation and non-pulmonary loss of the ^{13}C-atom. By determining m only for the population, this parameter

was considered a common feature of the study cohort. This simplification was required, and may be considered a conservative approach, to allow for robust individual detection of changes in β and k and thus reliable statistical comparison of t50_Ghoos between the two study arms. This approach was indirectly validated by the fact that it allowed the MRI confirmed detection of the pentagastrin induced delay in gastric emptying. The estimation of m to 71% lies within the upper range of previously reported values. The shorter cPDR curves of the pentagastrin arm caused some overestimation in the population value m, resulting in a larger offset for the calculated t50_Ghoos. However, the extent by which the offset in t50_Ghoos compared to t50_MV can be explained by the post-gastric metabolic processes or may be due to the applied standard analytical “Ghoos” method cannot be clearly deduced from this work. It may be suspected that it is also caused to a major part by the “Ghoos” method itself. Derivation of t50 by analyzing breath test data with other published methods, such as the method published by Bluck (28), did also lead to t50 values that correlated with t50_MV, but showed different systematic offsets in half emptying times. Due to these inconsistencies between analysis techniques and the lack of a gold standard, the collected ^{13}C-acetate breath test data was analyzed using the widely accepted “Ghoos” method that has been previously validated for liquid emptying.(21)

Pentagastrin infusion caused an increase in gastric secretion volume and also in meal emptying times (**figure 4A**). Various mechanisms that explain the observed decrease in meal emptying rate can be pursued: (1) Acidification of the duodenum with pH 1.0 – 2.7 induced a rapid increase in CCK plasma concentration which in turn is known to inhibit meal emptying.(29-34) (2) Neural reflexes involving acid-sensitive neurons that adjust delivery of acidic gastric content into the duodenum in order to balance the level of acid and hence to avoid mucosal lesions were triggered.(35) (3) Pentagastrin, irrespective of its secretion stimulating properties, may have had an effect on meal emptying by altering proximal gastric function, i.e. inducing relaxation of the fundus and increasing gastric wall compliance.(18) However, the given data did not allow for robust separation of gastric secretion and/or pentagastrin induced effects on meal emptying. To exclude the potentially overwhelming

effect of pentagastrin itself on motor inhibition, future studies on this topic should be designed using physiologic, non-pharmacologic secretion stimulation.

Considering that the range of changes in t50_Ghoos is comparable to the range of changes in t50_MV between pentagastrin and placebo infusion, it can be assumed that the endocrine pentagastrin effect is dominating over the intragastric secretion effect. Both methods detected a comparable prolonging effect of pentagastrin infusion on t50 with median [95% CI] differences in t50_Ghoos of 45 [28-84] minutes and t50_MV of 37 [28-52] minutes. Therefore, it may be speculated that pentagastrin affects the emptying process as a whole (and thereby in a more constant manner), whereas secretion might possibly have led to a partial or local effect in the stomach (and subsequent on the ^{13}C-marker). This would have resulted in a wider range of changes in t50_Ghoos between placebo and pentagastrin infusion and could also be another explanation for the missing correlation of AUC_SV_{60} and $cPDR_{60}$ (**figure 3C**). Additionally, the comparable prolonging effect of pentagastrin on t50_Ghoos clearly demonstrated that a pentagastrin infusion specific effect on post-gastric ^{13}C metabolism can be excluded.

Above findings suggest that $^{13}CO_2$ exhalation after ingestion of a ^{13}C-acetate labeled liquid test meal is not affected by the stimulated gastric secretion volume, but is rather reflecting the dynamics of meal or caloric emptying from the stomach.

In conclusion, this study represents another step towards the validation of the ^{13}C-acetate breath test for gastric emptying measurements. Even under potent pharmacological modulation of gastric secretion, the ^{13}C breath test can reliably detect intra-individual differences in meal half emptying times.

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Author contributions

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Critical revision of the manuscript: SK, AS, DM, WS, MFx

Statistics: SK, AS, DM

Study concept/design: OG, MF, WS, MFx

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Table

Table 1

Differences between pentagastrin and placebo derived half emptying times in minutes for breath test and MRI data.

Initials	Δt50_Ghoos (min)	Δt50_MV (min)
BF	32	53
CB	45	27
CH	0	37
DF	107	36
DS	95	42
JS	94	-5
MF	12	34
RB	12	68
RK	102	54
TB	89	59
TK	28	31

Figure legends

Figure 1: Individual Raw Data

A Individual PDR (% h^{-1}) and cPDR (%) data during infusion with pentagastrin (black) and placebo (grey). PDR was lower after 60 min of pentagastrin infusion in 8 of 11 volunteers; cPDR was lower in 7 of 11 volunteers.

B Individual MV (mL) and SV (mL) data during infusion with pentagastrin (black) and placebo (grey). MV decrease during pentagastrin infusion was lower in 7 of 12 volunteers. SV during pentagastrin infusion reached higher levels in 8 of 12 volunteers

Figure 2: Individual Fits

A Individual results for fitted cPDR (%, line) during infusion with pentagastrin (upper row) and placebo (lower row), overlaid on raw data (grey dots). Estimated population parameters with standard errors were: m = 71.34±1.04, log κ = −5.2±0.06, log β = 0.56±0.04.

B Individual results for fitted MV (mL, line) during infusion with pentagastrin (upper row) and placebo (lower row), overlaid on raw data (grey dots). Estimated population parameters with standard errors were: V_0 = 495.9±16.4, t_{empt} = 99.6±8.4.

Figure 3: Comparison of breath test and MRI derived data

A Correlation of t50_Ghoos (min) and t50_MV (min). The linear relationship was t50_Ghoos = (126 ± 19) min + (1.1 ± 0.3)*t50_MV, with r^2 =.67.

B The Bland-Altman plot of t50_Ghoos (min) and t50_MV (min). The plot shows an offset of 136 min for t50_Ghoos with limits of agreement of ±67 min.

C Correlation of AUC_SV_{60} (mL*min) and $cPDR_{60}$ (%). The linear relationship was $cPDR_{60}$ = (3566 ± 691) - (131 ± 80)*AUC_SV_{60}, with r^2=.11.

Figure 4: Differences in half emptying times (t50) between placebo and pentagastrin

A Individual differences in t50 and **B** median and 95% CI of the differences in t50 between pentagastrin (pgs) and placebo (plc), as assessed by breath test (t50_Ghoos) and MRI (t50_MV). Both figures highlight the wider range found for t50_Ghoos.